determination enigmatic at this time. Some puzzles remain with respect to comparisons between E16 and E14. We are additionally investigating the peptide-lipid behavior when Glu is introduced in position 12, at the peptide center.

3609-Pos Board B337

Influence of pH and Histidine Residues on Membrane-Spanning Helical Peptides

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Synthetic model peptides such as GWALP23 (acetyl-GGALW^{5}LALALALALALW^{19}LAGA-amide)provide a useful host framework for investigations of the influence of polar amino acids, for example histidine residues, within the hydrophobic core of a transmembrane helix. Importantly, membrane-spanning GWALP23 is quite sensitive to single-residue replacements, in part because the transmembrane helix exhibits only limited dynamic averaging of solid-state NMR observables such as the ^{2}H quadrupolar splitting (Biophys. J. 101, 2939). We inserted His residues into position 12 and/or 13 of GWALP23 (replacing either L12 or A13) and incorporated specific ^{2}H-Ala labels within the helical core sequence. Solid-state ^{2}H NMR spectra of GWALP23-H12 reveal a marked difference in peptide behavior between acidic and neutral pH conditions. At neutral pH, GWALP23-H12 exhibits a well-defined tilted transmembrane orientation in both DOPC and DLPC bilayer membranes. To prevent the acid catalyzed degradation of lipids, we employed ether-linked DOPC bilayers to observe the effect of low pH on the L12H mutant. Under acidic conditions GWALP23-H12 is highly dynamic and exhibits multiple states. Indeed, the multi-state behavior of GWALP23-H12, when His is charged between pH 1.5 and pH 3, resembles closely that of GWALP23-R12 at neutral pH (J. Am. Chem. Soc. 132, 5803). The dramatic change in the behavior of GWALP23-H12 indicates a pK_a value less than 3 for His near the center of a lipid bilayer. Investigations are in progress to chemically exchange the C2 imidazole hydrogen of His for deuterium in the peptide, toward a goal of enabling direct observation of the His ring by solid-state ^{2}H NMR over a range of pH and buffer conditions.

3610-Pos Board B338

Molecular Insight for the Effect of Lipid Raft on Thrombospondin-1 and Calreticulin Interactions

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Thrombospondin-1 (TSP1) binding to calreticulin (CRT) on the cell surface stimulates association of CRT with LDL receptor-related protein (LRP1) to signal focal adhesion disassembly and engagement of cellular activities (J. Biol. Chem. 275:36358-68, 2000; J. Biol. Chem. 277: 37219-28, 2002). Study demonstrated that lipid rafts are necessary for TSP1-mediated focal adhesion disassembly (J. Biol. Chem. 279, 23510-16, 2004), but the molecular mechanism of the phenomenon is still unknown. In this study, we investigated the interactions of a lipid bilayer and a lipid raft with CRT and TSP1-CRT complex and their effects on the structural changes of CRT and TSP1-CRT complex via atomically detailed molecular dynamics simulations. The lipid bilayer was modeled as a 1-palmitoyl-2-oleoyl-sn-glycero-3phosphocholine (POPC) bilayer with 1152 lipids. The lipid raft was modeled as a bilayer of POPC lipids mixed with cholesterol (CHOL) (40% of CHOL molecules in the lipid raft) or with both CHOL and sphingomyelin (SM) (the ratio of the number of POPC lipid, CHOL and SM is: 3:4:3). Results showed that TSP1 binding to CRT resulted in a more ''open'' conformation for CRT P-domain with respect to the CRT N-domain compared to that of single CRT in a lipid raft environment, but not in a POPC bilayer environment. Sphingomyelin enhanced the ''open'' CRT conformation by TSP1, which could expose the potential binding site(s) in CRT for binding to LRP1 to signal focal adhesion disassembly. Results also showed that micro- and mesoscopic properties of a lipid raft were significantly different from a POPC bilayer, which could also affect cell surface CRT interactions with TSP1. Results from this study provided molecular insight for the effect of lipid raft on TSP-CRT interactions and CRT-mediated focal adhesion disassembly.

3611-Pos Board B339

Analysis of the Molecular Organization of Lipoprotein-Associated Apolipoprotein E, an Anti-Atherogenic Protein

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Human apolipoprotein E3 (apoE3) is a 299 residue exchangeable apolipoprotein that has the ability to exist in lipid-free and lipoprotein-bound states. It is composed of an N-terminal domain bearing LDL-receptor binding sites and a C-terminal (CT) domain bearing high-affinity lipid-binding sites; the latter is also responsible for mediating cellular cholesterol efflux. ApoE plays a crucial role as an anti-atherogenic agent in atherosclerosis by virtue of its ability to mediate reverse cholesterol transport by promoting cholesterol efflux from macrophages, a process that leads to the initial formation of nascent discoidal high-density lipoproteins (nHDL). The objective of this study is to understand the molecular organization and conformation of apoE3 (1-299) and isolated CT domain (201-299) on reconstituted HDL (rHDL) and nHDL generated by macrophages. To accomplish this, we over-expressed and purified recombinant apoE3 and the CT domain bearing single Cys at selected sites. rHDL was prepared using unlabeled or pyrene-labeled single Cys variants and phospholipids, while nHDL was generated by exposing cholesterol-loaded J774 macrophages to unlabeled or labeled apoE bearing probes at defined sites. Lipid-associated apoE was obtained by density gradient ultracentrifugation. The relative organization of apoE molecules on rHDL and nHDL was followed by monitoring pyrene excimer fluorescence and by site-specific cross-linking studies, both complementary validators of spatial proximity at ~10 Å distance. The appearance of excimer emission and cross-linked dimers regardless of the location of the single Cys is indicative of pairs of apoE3 molecules oriented parallel to each other on the HDL to form a dimer. Based on our results, we propose that apoE molecules circumscribe a bilayer of lipids adopting a belt-like conformation. Our study offers mechanistic details of apoE interaction with lipids, which aids in understanding its role in cardiovascular disease.

3612-Pos Board B340

Dual-Color Fluorescence Cross-Correlation Spectroscopy of Reconstituted Protein-Membrane Systems

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Liposomes are commonly used as model membranes for studying the function of isolated membrane proteins. They are also used for reconstituting large membrane-associated protein complexes, such as the protein coats of intracellular transport vesicles. Here, we show examples of how dual-color fluorescence cross-correlation spectroscopy (or, more generally, fluorescence fluctuation spectroscopy) can serve to optimize in vitro reconstitutions and unravel protein-membrane interactions:

(A) The reconstitution of detergent-solubilized, purified membrane proteins into liposomes is a prerequisite for a myriad of experimental studies, but experimental conditions generally have to be determined on a case-by-case basis. Fluorescence correlation spectroscopy (FCS) distinguishes micelles, liposomes and aggregates in homogeneous and heterogeneous samples based on their different mobilities. Dual-color fluorescence cross-correlation spectroscopy (dcFCCS), an extension of FCS, additionally detects the co-localization of protein and lipid in these diffusing entities, facilitating the optimization process [1]. The principle is not limited to proteoliposome formation, but can be applied more generally to reconstitutions into small diffusing membrane entities, such as liposomes or nanodiscs.

(B) Studies of large, multi-subunit protein complexes, such as the COPII transport vesicle coat, require analysis of protein-lipid as well as protein-protein interactions. By virtue of their low perturbation, fluorescence techniques help optimizing complex reconstitution systems to obtain suitable preparations for cryo electron microscopy analysis of a complex structure [2], and help expanding the complexity of the reconstitution. The interaction of COPII components among each other and with model membranes are investigated.

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3613-Pos Board B341

Divalent Cation- and Cholesterol-Induced Perturbation in Lipid Lateral Organizations and Polyphosphoinositide-Protein Interactions

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Phosphatidylinositol 4,5-bisphosphate (PIP2) controls many important cellular events. A major challenge in understanding how PIP2 function in vivo is to define its physical state and lateral organization in cell membranes. The hypothesis that PIP2 forms nano-sized clusters in the presence of intracellular divalent cations by electrostatic interactions was examined in model membranes with or without cholesterol-mediated phase segregation. Additional studies show how

such membrane reorganization alters the effects of PIP2 on the target proteins gelsolin and DrrA.
Comparison between experimental and numerical phase diagrams suggests that a simplified electrostatic model can predict Ca^{2+}-driven formation of PIP2 clusters, but cannot account for the difference between Ca^{2+} and Mg^{2+} in condensing PIP2-containing membranes. Differences among Ca^{2+}, Mg^{2+} and multivalent polyamines in membrane condensing were revealed experimentally and related to differences in their dehydration enthalpies. Ca^{2+}-induced perturbation of PPI-protein interactions was assayed by monolayer insertion studies using a PI4P-binding protein, DrrA. Taking advantage of its unique biphasic effect on monolayer surface pressure, in which specific insertion can be isolated from non-specific adsorption, we show that Ca^{2+} suppresses the specific insertion of DrrA in a concentration-dependent manner. The perturbation of PIP2-protein interactions induced by cholesterol-mediated phase segregation was probed by measuring the inhibition of gelsolin's actin filament severing activity by PIP2-containing vesicles. Cholesterol-mediated phase segregation enhances the inhibition of gelsolin by PIP2, and this effect correlates with changes in membrane ordering. This result suggests that PIP2-protein interaction depends not only on global PIP2 concentrations but also on PIP2 lateral distribution without changes in lipid synthesis or degradation. The results of this work shed light on the links between PIP2 signaling and dynamic local response at the cell membrane/cytoskeletal interface.

3614-Pos Board B342
Dynamic Association and Dissociation of the Tumor Supressor PTEN to Model Membranes
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The tumor suppressor gene, *Phosphatase and tensin homolog deleted on chromosome 10* (PTEN) encodes a 403-amino acid protein that is mutated or deleted in a variety of human cancers. It binds to the plasma membrane to dephosphorylate the 3-position of phosphatidylinositol-3,4,5-trisphosphate producing phosphatidylinositol-4,5-bisphosphate ($PI[4,5]P_2$). PTEN is an agonist to PI 3-Kinase, thereby inhibiting the PI3K/Akt signaling pathway and controlling cell proliferation and survival. PTEN membrane association depends strongly on the composition and lateral distribution of the lipids in the membrane. Using kinetic methods, we aim to examine this multi-step process. Stopped-flow and steady state fluorescence experiments support our hypothesis that the association ofPTEN to the plasma membrane is synergistic; depending on both electrostatic interactions between the protein and anionic lipids like phosphatidylserine (PS) as well as specific binding to $PI(4,5)P_2$ to form a stable protein-membrane complex. In addition, our results suggest that other lipids found in the inner leaflet of the plasma membrane might participate in PTEN binding.
Furthermore, we use single molecule TIRF microscopy to determine the membrane binding and lateral diffusion constant of *wt*PTEN on supported lipid bilayers with differing lipid compositions. The information gained from these two techniques describes the dynamic and equilibrium behavior of *wt*PTEN binding to various binary and tertiary lipid compositions.

3615-Pos Board B343
A Tale of Two Domains: Different Roles of C1A and C1B Domains in PKC Interactions with Membranes
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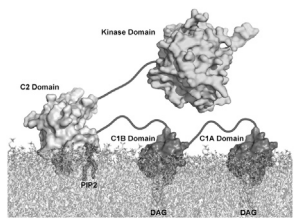
Protein Kinase C (PKC) is an important kinase family that controls major signaling pathways and has been associated with many diseases. Although conventional PKCs (for example, αPKC) are known to be activated upon two regulatory domains, C1A and C1B, binding to lipid activators in the membrane, key interactions and dynamics involved in the binding process are hard to study solely with experiments. With large-scale molecular dynamics simulations, we have studied the αPKC C1A and C1B domains in water and in various membranes, to understand their roles in PKC activation. Our simulations, for the first time, reveal the specific and non-specific interactions between the C1A/C1B domain and the membranes. Our results show that these tandem domains, although similar in structure, are different in terms of affinity to anionic lipids and response to lipid activators. Additionally, membranes are found to play a particular role to stabilize the C1A and C1B conformations. In general, our study suggests a PKC working model supported by further experimental evidence, which provides molecular-level understanding as a foundation for future investigations to modulate PKC activation through the C1A and C1B domains.



3616-Pos Board B344
Defining the Roles of Various Lysines and Arginines in Amot Lipid Binding
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One of the defining traits of cancerous cells is proliferation. We are focusing on the proliferation of mammary cells. As an adaptor protein, the Amot membrane binding event is key to the localization and sorting of proteins responsible for cellular differentiation, proliferation, and migration. The Amot coiled-coil homology domain (ACCH) is a lipid-binding domain responsible for affinity and binding to endothelial membranes. Our working hypothesis is that the ability to modulate Amot lipid-binding will lead to means to prevent ductal cell hyperplasia progression into breast cancer tumors. We will determine which residues are responsible for lipid-binding by changing positively charged lysine and arginine into uncharged or negatively charged amino acids. Those mutations which show a significant decrease in lipid-binding will then be used to determine their down-stream effects in human cells. The laboratory has screened approximately 40 of these mutations using a liposome binding assay. This assay mimics how the protein binds with the cell membrane by using an in vitro mixture of lipids similar to that seen in endothelial cells. Forster resonance energy transfer (FRET) was used to confirm significant decreases in lipid binding of ACCH mutants selected from the liposome binding assay, as energy transfer only occurs when the tyrosines in the protein and the Dansylated liposome are in close proximity to each other. Based on these two screens we have narrowed the list to seven mutants that have a significant decrease in lipid binding. Currently, FRET is being used to determine the lipid binding coefficient for each mutant of interest. Mutants deemed important from this study will then be transformed into human cells to study their effects on cell polarity, signal transduction, cell shape, and cellular proliferation.

3617-Pos Board B345
How Membrane Curvature Drives the Up-Concentration of N-Ras Proteins to Ordered Lipid Domains : Correlation of In Vivo and In Vitro Experiments with Mean Field Theory Calculations and Coarse Grain Simulations
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Sorting and trafficking of membrane-anchored Ras GTPases is critical for signaling and is believed to rely on their preferential portioning in ordered lipid-protein membrane domains (1). However studies in vitro have failed to quantify the preferential partitioning of full length Ras proteins into the liquid ordered phase(2), indicating that a physical principle underlying sorting of Ras is missing. We recently showed that lipidated proteins localize to highly curved membranes in vitro(3, 4). Here we provide a mechanistic insight on how membrane curvature can drive N-Ras sorting.
Combining the results of our in vitro assays, measurements on single vesicles, with in vivo studies, hypo-osmotic swelling of cells that flattens curved membrane regions, revealed that : a) N-Ras is preferentially recruited in areas of high membrane curvature and b) membrane curvature is the enabling factor underlying the selective partitioning of NRas in ordered domains. The combined readout of mean field theory calculations and coarse grain simulations provided a mechanistic insight on preferential partitioning in highly curved areas, via the changes in lateral pressure of the outer monolayer when curving an ordered versus a disordered membrane. In addition to providing the first biophysical sorting mechanism for Ras validated by both in vitro and in vivo measurements, our data indicate that membrane curvature may act as a generic cue underlying trafficking and sorting of multiple lipidated proteins.
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